THE IMPACT OF REPEATED WASHING ON RESIDUAL EFFICACY OF PYRETHROID LONG LASTING INSECTICIDAL NETS (LLINs) AGAINST DENGUE AND MALARIA MOSQUITO VECTORS

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PENGARUH PENCUCIAN TERHADAP EFEKTIVITAS RESIDU KELAMBU BERINSEKTISIDA PIRETROID LONG LASTING INSECTICIDAL NETS (LLINs) TERHADAP NYAMUK VEKTOR DEMAM BERDARAH DENGUE DAN MALARIA

ABSTRAK

Telah dilakukan evaluasi pengaruh frekuensi pencucian terhadap 3 macam kelambu berinsektisida "*Long Lasting Insecticidal Net*" (LLIN) dengan bahan aktif (b.a.) golongan insektisida pyretroid yaitu alfa-sipermethrin 0,2 g/m^2, deltamethrin 0,055 g/m^2 dan permethrin 1,0 g/m^2. Evaluasi dilakukan di laboratorium B2P2VRP Salatiga (pencucian kelambu berinsektisida digunakan metode standar WHO), terhadap nyamuk *Ae. aegypti* (vektor DBD) dan *An. aconitus* (vektor malaria). Evaluasi lapangan dilakukan di desa Tambangan, Kota Semarang, terhadap nyamuk *An. aconitus* dan pencucian kelambu dilakukan oleh kader kesehatan desa. Hasil evaluasi pengaruh pencucian menunjukkan bahwa daya bunuh tiga macam kelambu bertinsektisida LLIN terhadap *Ae. aegypti* dan *An. aconitus* di laboratorium dan terhadap *An. aconitus* di lapangan adalah sebanding. Di laboratorium tiga macam kelambu LLIN yang telah dicuci 5 kali masih efektif membunuh nyamuk *Ae. aegypti* dan *An. aconitus,* sedangkan kelambu LLIN telah dicuci 10 kali sudah tidak efektif terhadap nyamuk *An. aconitus*. Efektivitas kelambu LLIN (dengan insektisida alfa-sepermethrin, deltamethrin dan permethrin) di lapangan, setelah dicuci 9 kali oleh kader kesehatan desa, hanya kelambu dengan insektisida Deltamethrin (0,055 g/ m^2) masih efektif membunuh *An. aconitus* (kematian 82,47%).

Kata kunci : Kelambu berinsektisida, residu pencucian, nyamuk vektor

ABSTRACT

The influence of washing practices on the efficacy of pyrethroid Long Lasting Insecticidal Nets (LLINs) have been evaluated against mosquito vectors, e. i. *Anopheles aconitus* (malaria) and *Aedes aegypti* (dengue). The different active ingredient of pyrethroid insecticides studied, were alfa-cypermethrin 0,2 g/m^2, deltamethrin 0,055 g/m^2 and permethrin 1,0 g/m^2. The evaluation was conducted at of the Institute of Vector and Reservoir Control Research and Development laboratory, Salatiga and washing practices was done using WHO standard method. Field evaluatiuon against *An. aconitus* was done at Tambangan village, Semarang municipadity, were washing practices were conducted by the health village cader. The result showed that the influence of washing practices on the LLINs efficacy's againts *Ae. aegypti* and *An. aconitus* (Laboratory study) and *An. aconitus* (field study) were similar. Both study showed that all evaluated pyrethroid LLINs were effective to killed the mosquito vectors for less than 10 washes

Key words : Long Lasting Insecticidal Net (LLIN), washing resistance, mosquito vectors

INTRODUCTION

The most important malaria vector species tend to bite late at night, mosquito nets would be expected to protect against them effectively. Bed nets have been used to protect people from masquito bites. The traditional bed net is not effective sufficiently when it is torn and thus mosquitoes can enter through any holes. One way of overcoming this disadvantage is to treat bednets with an insecticide, preferably a pyrethroid, which is having rapid action against mosquitoes. Pyrethroids quickly knocked down and later kill mosquitoes upon contact.

Nets are generally treated by dipping them into an appropriate mixture of a pyrethroid formulation in water and drying them flat, preferably in the shade. A long-lasting insecticidal net (LLIN) is a factory-treated mosquito net that is expected to retain its biological activity for a minimum number of standard WHO washes and a minimum time under field conditions. Curently, a long-lasting insecticidal net would be expected to retain biological activity for at least 20 standard WHO washes under laboratory conditions and 3 years of recommended use under field conditions, as defined in the WHO guidelines (WHO, 2005).

Among the available control measures, vector control using mosquito nets has recently received some attention because it is a simple and an effective means of personal protection.

Impregnation of nets offered several advantages including extending the useful life of the net because treated nets are effective even when they are torn: providing a lethal resting site for mosquitoes even during the daytime when they are not used.

Insecticide impregnated bed nets (IBN) have been shown reduce malaria transmission (Binka *et al*, 1996 ; Lengeler 1998). These investigations were based on nets that were dipped in a water emulsion of a pyrethroid insecticide. Such nets lose their efficacy by repeated washes or after about 6-12 months of use, depending on the pyrethroid insecticide and product (Curtis *et al*, 1996; Binka *et al*, 1996; Vythilingam, 1996).

One major problem with the current combination of netting materials, insecticides and methods of treating nets is that regular washing quickly diminish the insecticidal effect of the nets. Malaria control programs often ask people using the nets, not to wash them between treatments. The frequency of washing nets varies. However, it is unreasonable to expect families to sleep inside nets that

have not been washed for a long time. It is therefore important that a combination of netting material, insecticide and method of treatment be developed that will allow normal washing without loss of effectiveness (WHO, 2006).

The objective of this study was to determine the impact of repeated washing on residual efficacy of pyrethroid Long Lasting Insecticidal Nets (LLINs) against malaria and dengue vectors, *Anopheles aconitus* and *Aedes aegypti.*

MATERIALS AND METHODS

Washing resistance

The resistance of an LLINs to washing was determined through standard bioassay carried out on nets washed at intervals required for regeneration, using the standard WHO method, dried and held at 30˚C. Bioassay was done after 0, 5, 10, 15 and 20 washes. Each bioassay was conducted just before the next wash (WHO, 2005). The efficacy of pyrethroid mosquito nets LLINs were evaluated under laboratory and field condition.

Laboratory evaluation

Determination of time period required for full regeneration of the Long Lasting Insecticidal Nets (LLINs) upon after washing practices. The evaluation was conducted in the laboratory against malaria vector *An. aconitus* and DHF vector *Ae. aegypti.*

Field evaluation

Determination of time period required for full regeneration of the Long Lasting Insecticidal Nets (LLINs) after washing practices which was done monthly by the village caders. Evaluation residual efficacy of LLINs was carried out against rice fields malaria vector *An. aconitus.*

Both studies were on January – August 2008 conducted, in the Institute for Vector-Reservoir Control Research and Development (IVRCRD), Salatiga Municipality and in the rice fields' areas of Tambangan Village, Mijen Subdistrict, Semarang Municipality, Central Java Province, using bioassay test method (WHO, 2005).

LABORATORY EVALUATION

1. Materials:

   Bioassay test kits, sucking tube, plastic cups, cotton wool, rubber band, glucose solution, powder soap, alcohol, Three types of pyrethroids Long Lasting Insecticidal Nets (LLINs) evaluated. The nets were impregnate with three different active ingredient (a.i), of pyrethroid insecticides : Alphacypermetrin (0,2

g/m^2), Deltametrin 0,055 g/m^2 and Permetrin (1,0 g/m^2).

Each LLIN type was selected and cuted in piece for 25 cm X 25 cm (three pieces for each mosquito net's side). Bioassay tests of LLINs were done for: none (before washing), 5, 10, 15 and 20 times washes**.** Female mosquitoes *An. aconitus* or *Ae. aegypti,* 3-4 days old, glucose fed condition (colonies maintained in the laboratory of the IVRCRD) were used for evaluation.

2. Methods

a. Net washing **(**WHO, 2005)

The net samples (25 cm X 25 cm) of each LLINs type was individually introduced into blue cap bottle (1000 ml volume), containing 500 ml of deionized water and 2 g/l soap ("Savon de Marseille" recommended the standard soap), pH 10-11, added just before and fully dissolved. Bottles were shaked immediately for 20 minutes at 155 movements per minute. The samples were removed and rinsed twice for 20 minutes in clean deionized water in the same shaking conditions as stated above. Nets were dried at room temperature, wraped in aluminium foil and stored at 30^{o}C in the dark between washes.

b. Bioassay test

Five (susceptible strain) sugar fed, 2-4 day old tested mosquitoes, *An. aconitus* and *Ae. aegypti*, were exposed to netting materials (25 cm X 25 cm) for 3 minutes, under standard WHO cones. After sixty minutes of contact the *kocked down* (KT) mosquito were camted and kept under observation for 24 hours in laboratory, to calculate mortality. At least 180 mosquitoes were tested on each net sample (4 sides, three replicates, 15 mosquitoes/cone). Tested mosquitoes were also exposed to untreated nets, used as control/UTC. They were then exposed for 3 minutes in the exposure cone, gently taken out after exposure and kept into the holding plastic cups to determine the mortality after 24 hours holding in the laboratory, with access to sugar solution. All bioassay tests were conducted in the laboratory maintained at room temperature 25-29˚C and the humidity of 68-92%.

FIELD EVALUATION

1. Materials:

Bioassay test kits, sucking tube, plastic cups, cotton wool, rubber band, glucose solution, powder soap, alcohol, three type of pyrethroid

LLINs sample, as stated on the laboratory evaluation.

Nine village houses (similar in condition) were chosen for the study. Three other selected houses were used for untreated insecticide mosquito nets which were used as untreated control/UTC. Each LLIN type was provided to be used by the people, in each selected house (three replicates for each LLIN type).

2. Methods

a. Net washing

Net samples measure (180x160x150 cm) were distributed to nine selected families in the study village (two units for each family). A total of 18 unit LLINs (6 unit for each type), were evaluated for the study. The nets were individually washed by the village cader, using well water and ordinary soap which is normally used by the villagers for clothes washing. Ten grams of washing soap powder added into the bucket which was provided with 10 liters of well water. Washing method applied as commonly done by the villagers and rinsed twice using well water. The nets were dried at room temperature and heavy shade place (not direct to the sun rise), before fixed at the sleeping bed. Washing practices of the LLINs were conducted biweekly, before the bioassay test performed. Evaluations were done for 10 times (during the study), before washed and (1-9) post washed practices. Evaluation was terminated when tested mosquito's mortality was less than 80%.

b. Bioassay test

Five female susceptible strain mosquitoes (glucose fed, 3-4 days old) *An. aconitus* were exposed to the mosquito nets (which have been regularly used by the villagers). Exposure was done for 3 minutes, under WHO standard cones. Same bioassay test method of laboratory evaluation was applied for this evaluation.

DATA ANALYSIS

The data was analyzed descriptively and also by using varian analysis of tested mosquito mortality *An. aconitus* and *Ae. aegypti* after bioassay evaluations.

RESULTS

Laboratory evaluation

The results of the bioassay test on *Ae. aegypti* carried out in the laboratory for all evaluated LLINs (Alphacypermethrin 0,2 g/m^2, Deltamethrin 0,055 g/m^2 and Permethrin 1,0 g/m^2), for unwashed and after repeated washing, presented on Table 1 and Figure 1.

The mortality of *Ae. aegypti* on unwashing of all evaluated nets were 100%. The mortalities on LLINs of Alphacypermethrin 0,2 g/m^2, Deltametrin 0,055 g/m^2 and Permethrin 1,0 g/m^2, were respectively dropped to 68,44%, 60,22% and 55,56% at the 10th washes.

Table1. Mortality (%) of tested mosquito *Ae. aegypti* after exposured on the LLIN (Laboratory evaluation).

LLINs	Number of washing practices				
	None	5	10	15	20
Alphacypermethrin	100	93,78	68,44	52,00	30,52
Deltamethrin	100	95,33	60,22	44,89	24,81
Permethrin	100	90,00	55,56	37,11	21,56

The influence of washing practices of (LLINs) Alphacypermetrin (0,2 g/m^2), Deltametrin (0,055 g/m^2) and Permetrin (1,0 g/m^2), against malaria vector *An. aconitus* in the laboratory, presented on Table 2 and Figure 2. The efficacy of evaluated LLINs Alpha-cypermetrin (0,2 g/m^2) and Deltametrin (0,055 g/m^2), were killing *An. aconitus* effectively for 10 washes. The average mortalities for 10 washes for Alphacypermethrin and Deltamethrin were 87,78% and 86,89% (> 80%). In addition, Permethrin LLIN was only effective for less than 10 washes.

Table 2. Mortality (%) of tested mosquito *An. aconitus* after exposured on the LLIN (Laboratory evaluation).

LLINs	Number of washing practices				
	None	5	10	15	20
Alphacypermethrin	100	89,33	87,78	65,11	30,50
Deltamethrin	100	99,56	86,89	62,44	32,56
Permethrin	100	81,56	58,00	47,56	22,83

Field evaluation

The influence of washing practices of (LLINs) Alphacypermethrin (0,2 g/m^2), Deltamethrin (0,055 g/m^2) and Permethrin (1,0 g/m^2), against malaria vector *An. aconitus* field evaluation, presented on Table 3 and Figure 3.

Table 3. Mortality (%) of tested mosquito *An. aconitus* after exposured on the LLIN (Field. evaluation).

Number of washing practices	Percent (%) Mortality of tested mosquito		
	Alphacypermethrin	Deltamethrin	Permethrin
None	100	100	100
1	99,26	100	93.70
2	100	92.59	100
3	100	100	100
4	99.63	99.75	100
5	94.88	96.79	88.27
6	94.69	94.69	86.79
7	87.04	91.23	81.91
8	81.79	85.43	78.70
9	78.15	82.47	73.52

Mortality of *An. aconitus* post exposed on pyrethroids LLINs Alphacypermethrin 0,2 g/m^2, Deltamethrin 0,055 g/m^2 and Permethrin 1,0 g/m^2, were respectivelly effective for 8, 9 and 7 washes, and the average mortalities were 81.79%, 82.47% and 81.91% respectivelly (> 80% mortality).

DISCUSSION

LABORATORY EVALUATION

The evaluated mosquito nets of long lasting insecticidal nets (LLINs) made of polyester multi-filament 75 denier, which were impregnated with a.i Alphacypermethrin 0.2 mg/m^2, and Deltamethrin 0,055 g/m^2, effective to kill *Ae. aegypti* for less than 10 washes.

The percentage mortality of *Ae. aegypti* exposed to Alphacypermethrin 0.2 g/m^2, Deltamethrin 0.055 g/m^2 and permethrin 1.0 g/m^2 after 5 washes were respectively 93.78%, 95.33% and 90.0%, while for *An. aconitus* with mortality as 89.33%, 99.56% and 81.56%. After the nets had been washed 10 times, the percentage mortality of *Ae. aegypti* exposed to Alphacypermethrin 0.2 g/m^2, Deltamethrin 0.055 g/m^2 and Permethrin 1.0 g/m^2 was 68,44%, 60,22% and 55,56%, while for *An. aconitus* the mortality was 87,78%, 86,89% and 58,0% (Table 1 and 2, Figure 1 and 2).

Mortality of *Ae. aegypti* exposed to all evaluated LLINs was dropped below the cut off value of 80.0% after 10 washes. While mortality of *Ae. aegypti* exposed to Permethrin 1.0 g/m^2 LLIN was dropped below the cut off value, after 10 time washes, it was 55,56%.

After 10 washes, mortality of *An. aconitus* exposed to all evaluated LLINs was dropped below the cut off value of 80.0% (Table 2).

Laboratory study of Interceptor® LLIN (Alphacypermethrin, with the target dose of 0,2 g/m^2 of polyester fabric) was reported by WHO (2007) as good performance against *An. gambiae* in term of efficacy and was resistance. This net met the WHOPES phase criteria of KD affect above the 95% after 20 washes. Mortality dropped below the cut-off value of 80% after 20 washes.

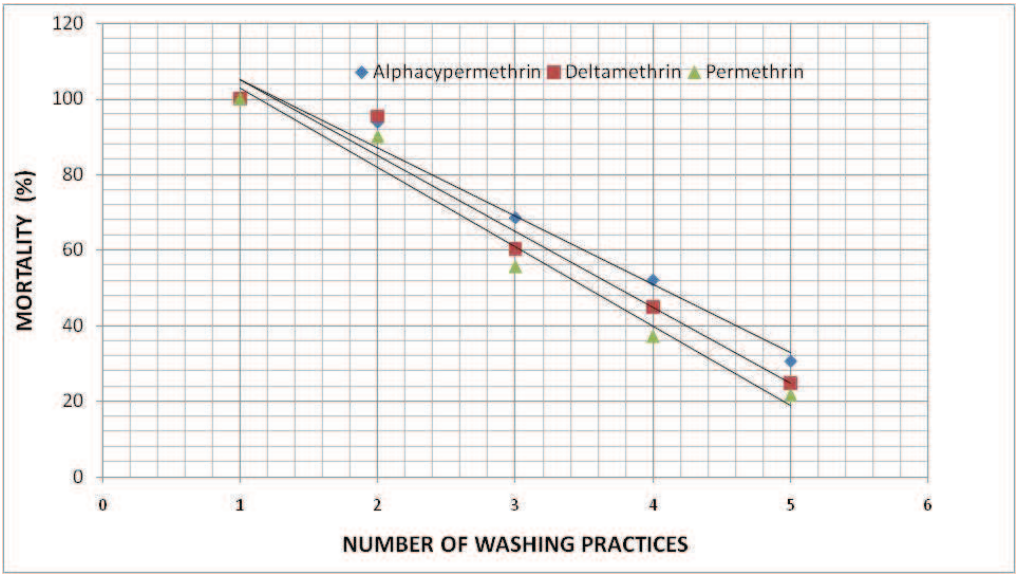
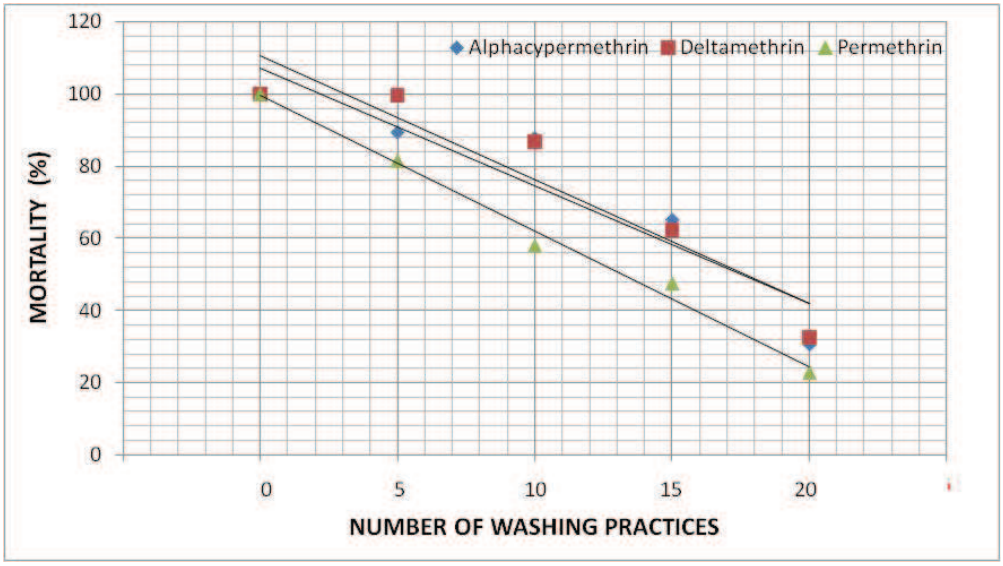


Figure 1. Reduction the efficacy of LLINs against *Ae. aegypti* in relation to washing practices frequency in the laboratory.

The efficacy of Olyset LLIN (Deltamethrin 0,055 g/m^2) was studied in Malaysia. The study revealed that the percentage mortality of *An. maculatus* exposed to Olyset, nylon multifilament and polyethylene nets after 15 washes with water was 95%, 83% and 26% respectively, while for *Ae. aegypti* with mortality of 100%, 91.7% and 81.7%, respectively. After the nets had been washed four times with soap and water, the percentage mortality of *An. maculatus* exposed to Olyset, nylon and polyethylene nets was 86.7%, 80.3% and 3.3% respectively. While for *Ae. aegypti* the mortality was 90.3%, 50% and 5% respectively (Vythilingam 1996).

This study revealed that mortality of *Ae. aegypti* exposed to all evaluated LLINs impregnate with pyrethroids insecticide such as: (Alphacypermethrin 0.2 g/m^2, Deltamethrin 0.055 g/m^2 and Permethrin 1.0 g/m^2), were remained at closed to 80% for less than 10 washes (Table 1, Figure 1).



Figue 2. Reduction of the efficacy of LLINs against *An. aconitus* in relation to washing practices frequency in the laboratory

FIELD EVALUATION

The average mortality of *An. aconitus* exposed on three type of LLINs Alphacypermethrin 0.2 g/m^2, Deltamethrin 0.055 g/m^2 and Permethrin 1.0 g/m^2 after 9 washes were respectively 78.15%, 82.47% and 73.52%. These results mean that the efficacy of LLINs Alphacypermethrin 0.2 g/m^2, Deltamethrin 0.055 g/m^2 and Permethrin 1.0 g/m^2 were respectively effective for 8, 9 and 7 washes, due to the mortality of tested mosquito of *An. aconitus* that dropped below the cut off value of 80.0% (Table 3, Figure 3)

Varian analysis (one–way, multiple comparation) showed that the influence of washing practices of the efficacy evaluated LLINs: Alphacypermetrin 0,2 g/m^2, Deltametrin 0,055 g/m^2 and Permetrin 1,0 g/m^2, were found similar.

Mortality of *An. aconitus* after exposed on all evaluated LLINs before washing was not significantly different ($P < 0.05$), compared to 1, 2, 3 and 4 times washes.

The efficacy of three LLINs tested for 5 and 6 washes were not significantly different ($P < 0.05$). Same results were also found for 7, 8 and 9 washes, which were not significant different ($P < 0.05$).

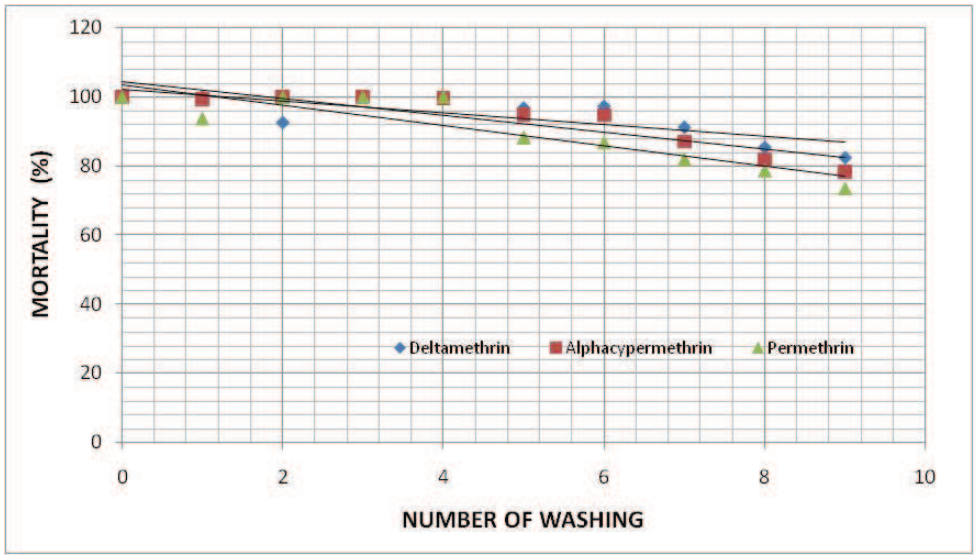


Figure 3. Reduction the efficacy of LLINs against, *An. aconitus* in relation to washing practices frequency in the field

Both studies, Laboratory evaluation (conducted against *Ae. aegypti* and *An. aconitus*) and field evaluation against *An. aconitus*, showed that the net samples were only effective at least for less than 10 washes (mortality more than the cut off value of 80.0%). These results were actually less than WHO recommendation

that long-lasting insecticidal net (LLINs) should be expected to retain biological activity for at least 20 standard WHO washes under laboratory conditions. In addition to that, the LLINs at least suppose to be used for 3 years under field conditions, as defined in the WHO guidelines (WHO, 2005 and 2006).

SUMMARY

The effectiveness of pyrethroids long lasting insecticidal nets (LLINs), impregnated of Alpha-cypermethrin 0.2 g/m^2, Deltamethrin 0.055 g/m^2 and Permethrin 1.0 g/m^2 against *Ae. aegypti* and *An. acontius* both in the laboratory and *An. aconitus* in the field evaluations were only less than 10 washes.

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